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Brief Communication

De novo PIK3R1 gain-of-function with recurrent sinopulmonary infections, long-lasting chronic CMV-lymphadenitis and microcephaly

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ABSTRACT

PIK3R1 (phosphoinositide-3-kinase, regulatory subunit 1) gain-of-function has recently been described in patients with recurrent sinopulmonary infections, chronic CMV−/EBV-infections, lymphoproliferation, and hypogammaglobulinemia. Here we report a 15-year-old boy with treatment refractory CMV lymphadenitis, severe combined immunodeficiency, microcephaly and a severe developmental defect of Th17 cells. To avoid poor outcome, hematopoietic stem cell transplantation (HSCT) was performed. Subsequently, whole exome sequencing revealed a *de novo* heterozygous G-to-C mutation (chr5: 5:67,589,663: G > C) at the splice donor site of the *PIK3R1* gene. Our data suggest that *PIK3R1* gain-of-function leads to developmental defects in helper and regulatory T-cell subsets, the latter expanding the immunological features of *PIK3R1* gain-of-function. T-cell subsets play a critical role in the regulation of immune response against infectious agents and of autoimmunity and thus may be particularly accountable for the clinical phenotype of affected patients.

1. Introduction

The co-occurrence of sinopulmonary infections, lymphoproliferation, viremia due to cytomegalovirus (CMV) and/or Epstein–Barr virus (EBV), deficiency of naïve T cells and over-representation of senescent T cells has recently been described as a novel human immunodeficiency termed "PASLI disease" (p110δ-activating mutations causing senescent T cells, lymphadenopathy and immunodeficiency; APDS) [1]. It is caused by dominant-activating gain-of-function germline mutations in the phosphatidylinositol-3-OH kinase (PIK3CD) p110δ subunit with hyperactivation of mTOR and heightened AKT phosphorylation. A similar "PASLI-like" disease also termed "activated PI3Kδ syndrome 2 (APDS2)" caused by heterozygous splice site mutations in *PIK3R1*, which encodes the p85α, p55α, and p50α regulatory *PIK3* subunits, has subsequently been described [2]. These patients likewise suffer from recurrent sinopulmonary infections, lymphoproliferation and hypogammaglobulinemia, and exhibit hyperactive PI3K signaling. Up to now, two different groups reported on this novel immunodeficiency disease characterizing 8 patients from 5 families with three different heterozygous gain-of-function mutations (G > C (n = 5), G > A (n = 2) or G > T (n = 1) at chr.5:67589663) affecting the same splice site in *PIK3R1* [2,3]. Whereas *PIK3R1* hyperactivating mutations seem to lead to defects in lymphoid homeostasis and B-cell Ig class switching, it is particularly unclear whether defects in helper and regulatory T-cell subsets may contribute to the disturbed immune function and finally shape the clinical phenotype of affected patients [4].

2. Patient

2.1. Medical history

Here we report a *PIK3R1* gain-of-function mutation in a 15-year-old boy of non-consanguineous parents presenting with generalized peripheral lymphadenopathy, chronic CMV infection, and severe developmental defect of Th17 cells augmenting the previously reported phenotype in *PIK3R1* mutations [2,3].

His history was remarkable for two hospital admissions, for pneumonia at age 20 months and mastoiditis at age 4 years with fever, and

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recurrent otitis media, adenoidectomy/tonsillectomy. The boy was first evaluated because of generalized peripheral lymphadenopathy combined with a slight hepatomegaly at the age of 7 years. Except low weight, height and microcephaly (all below 3rd percentile), there were no other relevant findings. IgM was slightly elevated, IgD clearly increased, and IgG_4 not detectable. Serology showed past infection with EBV and both IgM and IgG positive for CMV with a viral load of 2150 copies/ml. A lymph node biopsy showed florid follicular hyperplasia with a CMV viral load of 240,000 copies/mg tissue. Double negative alpha/beta positive T cells were within the normal range (0.5%), whereas gamma/delta positive cells were remarkably increased (20%).

The boy showed an impressive clinical response to treatment with Ganciclovir. Clearance of CMV viremia succeeded within two weeks of treatment and lymphadenopathy regressed. The treatment was changed to Valganciclovir. Follow-up blood samples were intermittently positive for low level of CMV antigen but no quantification of viral load was done at that time.

Due to recurrence of lymphadenopathy, symptoms of upper airway obstruction, and weight loss while under Valganciclovir, the boy was reevaluated at the age of 9 years. A non-specific febrile illness preceded the onset of symptoms; the lymphadenopathy was progressing long after resolution of the acute illness. Lymph node biopsy again showed hyperplasia with positive immunohistochemical staining for CMV. Steroid treatment was commenced and Valganciclovir continued. The clinical response was rapid with halving of palpable glands in five days. CMV remained detectable intermittently at a low titer. Steroid treatment was continued for two years with subsequent remission of the lympho-proliferative disease for the following 2¼ years.

At the age of 14 ½ years the boy once more presented with massive lymphadenopathy at all peripheral lymphatic areas (as shown in Supplementary material Fig. S1. Additional data on the clinical presentation are given in Supplementary material Table S1). Lymph node biopsy again showed CMV lymphadenitis (as shown in Supplementary material Fig. S2). CMV DNA in the serum was negative. Immunoglobulin levels were within the normal range. Treatment with Ganciclovir and immunoglobulins was initiated and subsequently switched to Valganciclovir. After discontinuation of Valganciclovir, the CMV lymphadenitis recurred but did not sufficiently respond to antiviral therapy anymore. The boy was transferred to our institution for further diagnostics and hematopoietic stem cell transplantation (HSCT).

2.2. Immunological features

Immune phenotyping showed remarkable circulating lymphocyte populations. While the T-cell count was within the normal range, naïve and memory $CD4^+$ T cells were decreased and both naïve and memory $CD8^+$ T cells increased with a very low $CD4^+/CD8^+$ ratio (0.2). Gamma/delta T cells were within the normal range, although they have been reported to have amounted to 20% in peripheral blood at the age of 7 years.

The B-cell count was clearly decreased with a paucity of class-switched memory B cells (Table 1). As IgG_4 was reported to be not detectable in early childhood, defects in the secretion of class-switched immunoglobulins were suggested. However, when the patient was referred to our department, serum concentrations of immunoglobulin G was decreased but concentrations of IgA, IgM and IgG subclasses were normal.

Addressing lymphocyte function in more detail, we assessed the in vitro proliferation activity of the patient's peripheral blood lymphocytes that showed an inadequate stimulation after exposure to phytohemagglutinin (PHA), anti-CD3 (OKT3), and Pokeweed Mitogen (PWM) (Fig. 1b) and a poor reactivity to T-Toxoid, PPD, and Candida both compared to healthy controls. Serum antibody titers to tetanus and diphtheria were within the lower limit.

Table 1
Immunophenotype analyses of leukocytes of the peripheral blood.

	05/2014	08/2014	Reference value	
Leukocytes/μl	8500	8100	5900	(4800–7400)[c]
Lymphocytes [%]	35.6	19	34.3	(27.0–44.1)[c]
Lymphocytes absolute	3026	1539	2100	(1500–2700)[c]
T cells [%]	88	85	68	(52–90)[a]
$CD4^+$ [%]	10	7	36	(20–65)[a]
$CD8^+$ [%]	69	76	24	(14–40)[a]
$CD45RA^+CD4^+$ [%]	28	2	62	(49.4–71.9)[c]
$CD45RO^+CD4^+$ [%]	70	97	37.5	(27.3–49.8)[c]
$CD45RA^+CD8^+$ [%]	56	48	64.3	(48.6–87.5)[c]
$CD45RO^+CD8^+$ [%]	43	65	28	(11.7–42.9)[c]
$TCR\alpha\beta^+CD4^-CD8^-$[%]	1.2	0.5	2	(0.54–6)[a]
$TCR\gamma\delta^+$ [%]	13	12	6	(2–17)[a]
$CD45^+CD127^+$ (IL7RA) [%]	13	68		
$CD45^+CD132^+$ (IL2RG) [%]	56	47		
B cells [%]	2	3	14	(9.1–21)[b]
$CD20^+CD40^+$ [%]	2	3		
IgD^+CD27^- [%]	62	59	14	(9.1–21)[b]
IgD^+CD27^+ [%]	3	10	1.42	(0.85–2.53)[b]
IgD^-CD27^+ [%]	1.7	3	1.2	(0.4–3.25)[b]
Natural killer cells				
$CD56^+CD3^-$ [%]	5.4	5	14	(4.0–51)[a]
$CD56^+CD3^+$ [%]	13	19	3	(0.64–15)[a]
Granulocytes				
$CD66b^+CD49d^-$ [%]	62	70		
$CD66b^+CD49^+$ [%]	5	2.5		
Other				
$CD18^+$ [%]	98	99		
$CD14^+$ [%]	5	3		
Immunoglobulins, g/l				
IgG	577			716–1711
IgG_1	361			370–910
IgG_2	130			110–485
IgG_3	50.3			24.0–116.0
IgG_4	11.2			5.2–196.1
IgA	99			47–249
IgM	109			15–188

[a] Schatorjé EJ. et al., 2012, Scand J Immunol., 75(4):436-44 (Pediatric Reference Values for the peripheral T-cell compartment.)
[b] Huck K et al., 2009, Clin Immunol, 131(1):50-9 (Memory B cells in healthy and antibody-deficient children.)
[c] van Gent R. et al., 2009, Clin Immunol., 133(1):95-107 (Refined characterization and reference values of the pediatric T- and B-cell compartments.)

3. Results and clinical course

3.1. Hematopoietic stem cell transplantation

Although the underlying genetic defect was not known at that time hematopoietic stem cell transplantation (HSCT) was opted to avoid poor outcome with regard to the patient's clinical presentation with treatment refractory CMV lymphadenitis and severe combined immunodeficiency with low CD4/CD8 ratio and lacking memory B cells. As no HLA-matched unrelated donor could be found, maternal haploidentical $TCR\alpha\beta-$/CD19-depleted peripheral blood stem cell transplantation was performed at the age of 15 years. Conditioning regimen comprised thymoglobulin, fludarabine, thiotepa, and targeted busilvex. Ciclosporin was given for Graft-versus-Host-Disease (GvHD) prophylaxis from d-1 and mycophenolatemofetil from d-0. Leukocyte engraftment was observed on d + 13. The patient was discharged on d + 61 and is now, 260 days after HSCT, well.

3.2. Whole exome analysis

Whole exome analysis revealed a heterozygous G-to-C mutation (chr5: 5:67,589,663: G > C; defined according to Ensembl (v70)) at a splice donor site of the *PIK3R1* gene, recently described by Deau et al. [3] and Lucas et al. [2]. The PIK3R1 splice site mutation causes skipping of an exon, corresponding to loss of amino acid residues 434–475 in the inter-SH2 domain. Sanger sequencing confirmed that the heterozygous